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THE AXIAL GRADIENTS IN HYDROZOA.

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IV. AXIAL GRADATIONS IN RATE AND AMOUNT OF REDUCTION OF POTASSIUM PERMANGANATE IN VARIOUS HYDROIDS AND MEDUSÆ.

In an earlier paper (Child, '19*a*) attention was called to a method of rendering directly visible the physiological axial gradients in many of the simpler organisms and earlier developmental stages through the differences in rate and in total amount of reduction of potassium permanganate by the protoplasm of different regions and levels. Since the reduced permanganate (MnO_2 or other oxides) colors protoplasm brown to opaque black according to amount of deposit, differences in rate or amount of reduction in different regions appear as differences in depth of coloration or in degree of opacity.

In the paper referred to, the results of work with permanganate on various animal and plant species are briefly described and it is pointed out that the gradients in rate and amount of permanganate reduction correspond to the physiological gradients demonstrated by means of the susceptibility method and various other methods.

MATERIAL AND METHOD.

The present paper records observations made on the reduction gradients, as they may for convenience be called, of various species of hydroids and medusæ. The work was done at the Puget Sound Biological Station chiefly during the summers of 1918 and 1919 and I am indebted to the director for the privileges of the station. The data concerning the hydroids were obtained chiefly from four species, *Bougainvillea mertensi*, *Gonothyræa clarki*, *Obelia borealis* and *O. longissima:* but observations on rate of reduction were also made on several other campanularian species. Young hydroids were grown in the laboratory from the eggs of *Gonothyræa* and the hydromedusa *Phialidium gregarium*

and the physiological gradients have been followed through all developmental stages by various methods, but consideration of the embryological data is postponed to a later paper. Among the hydromedusæ four species, *Phialidium gregarium*, *Mitrocoma discoides*, *Æquorea cærulescens* and *Sarsia rosaria* constituted the chief material for the work with permanganate, but observations were made as opportunity offered on several other forms, *Stomatoca*, *Obelia* and several undetermined species.

The experimental procedure consists merely in immersing the species to be examined in a solution of $KMnO_4$ in sea water: For observations on the color gradients resulting from differences in rate of reduction concentrations ranging from $m/1000$ $KMnO_4$ down to $m/10{,}000$ or even less have been used in most cases. Higher concentrations than $m/1000$ may of course be used, but in such concentrations the color of the solution is so deep that the animals must be removed to water for observation. Moreover, the differences in rate of reduction and staining are more distinct in the lower than in the higher concentrations because the latter kill all parts almost at once in spite of the differences in physiological condition. Differences and gradients in total amount of reduction are determined by permitting the reduction to proceed to completion in excess of permanganate. Under these conditions most organisms become opaque black and no differences in depth of color can be seen, but in certain small organisms, embryonic stages, or regions of body, in which the thickness of protoplasm or cell mass is not too great, the color gradient resulting from differences in amount of MnO_2 deposited is directly visible. Many other forms which are opaque black in water may be made more or less translucent by hardening and gradual dehydration in alcohol and clearing in an oil, and if desired mounting in balsam. The axial gradients in blastulæ and other early embryonic stages of various species, as well as in the adults of various small forms, can be seen with great distinctness in such preparations. Larger organisms or regions which reduce relatively large amounts of permanganate may remain opaque even after clearing. In fact, the method is chiefly of value for small, more or less translucent organisms, but for these it gives very definite, uniform and beautiful results.

I have made no attempt to determine at all exactly the length of time necessary for completion of the reaction in given concentrations but have merely made certain that length of time was sufficient for the purpose. Usually preparations of this sort remained 24–48 hours in a solution of $m/1000$ or higher. My observations indicate, however, that in small organisms, such as hydroids, and in embryonic stages the reaction is in most cases complete in much shorter time, *e.g.*, 2–4 hours in $m/1000$.

All concentrations of $KMnO_4$ high enough to give any appreciable deposit of the oxide on or in the protoplasm are highly toxic. Even in $m/20{,}000$ ciliary activity ceases within a short time, ranging from a few seconds to about one minute in forms examined and traces of the color begin to appear on the external surfaces within two or three minutes. Except in those concentrations in which cytolysis and disintegration take place it is impossible to determine exactly when death occurs. Undoubtedly it occurs before reduction and staining proceed very far, for alteration in aggregate condition, apparently a coagulation, can often be observed in the cells in early stages of coloration.

Certain precautions necessary in the use of the permanganate may be noted. Certain low concentrations produce cytolysis and disintegration in many, perhaps in all, organisms. In such cases susceptibility gradients corresponding to those observed with other agents and to the reduction gradients appear. In concentrations producing disintegration the disintegrated mass may show more or less distinct gradients in rate or amount of reduction, provided it holds together, but commonly cytolysis is followed by loss of continuity and by distribution of the cell substance through the water. In higher concentrations the reduction gradients appear without disintegration.

In order to avoid irregularities in staining it is necessary to agitate the solution frequently or to move the organisms about in it. Reduction of the permanganate at the surface of the organism often occurs much more rapidly than its diffusion, and a zone of low concentration of permanganate may appear about the organism, particularly about its more rapidly reducing regions. Parts in contact with the glass or lying near other

reducing parts soon show a retardation in staining in consequence of decrease in concentration of permanganate in the region about them. For these reasons the volume of solution used should be large as compared with the volume of protoplasm and uniformity of concentration at all points of the protoplasmic surfaces should be maintained by continuous or frequent agitation.

One difficulty exists in the technique of permanent preparation of permanganate material either for whole mounts or for sections. All clearing agents used thus far remove more or less rapidly the black or brown deposit in the protoplasm, the MnO_2 being apparently soluble in or reacting in some way with the oils. This disappearance of color also occurs in Canada balsam. In the samples of clove oil used the disappearance of the color is rapid, *e.g.*, hydranths may fade from opaque black to light uniform yellow in two or three days. In cedar oil the fading occurs much more slowly and may be a matter of weeks. In the course of a study of permanganate preparations of larval stages of echinoderms Mr. A. E. Galigher has found that with rapid preparation sections could be obtained without appreciable loss of color, but that in balsam the color gradually disappeared. Small organisms, larval stages, etc., can be made sufficiently translucent by dehydration and clearing so that the differences in total amount of reduction are clearly visible and persist at least for a day or two, or with some clearing agents much longer.

It is scarcely necessary to point out that differences in thickness of the layer of protoplasm through which the light is transmitted may appear as differences in depth of staining. A hydroid stem of larger diameter, for example, may appear to be more deeply colored than a stem of smaller diameter and in such cases it is often quite impossible to determine whether the difference is real or apparent. Such difficulties arise chiefly in connection with preparations for total amount of reduction rather than with those for rate of reduction, for in the latter reduction and coloration occur first on the surface and progress inward, and the differences in rate of staining can usually be seen in early stages on the external surface of the protoplasm quite independently of its thickness.

Thus far an exact quantitative record of the differences in rate of staining has not been attempted because of the difficulties involved. It is difficult to determine exactly the moment when the staining begins in a certain region and although the regional differences in depth of color are very marked, even in forms as small as the hydroid planula or sea urchin blastula and gastrula, any adequate measure of this difference is not readily obtained. Photomicrographs can undoubtedly be made of stages of staining or of the cleared preparations, but in any case are little better than figures, and graphic methods are much less readily applied than in case of disintegration experiments (Child, '15, Chaps. III.–VII.). In the present paper description is supplemented by a few diagrammatic figures in which the regional differences are indicated at some stage of coloration by degrees of shading.

Reduction Gradients in Hydroid Colonies.

Before describing the details it may be said that in general both the rate and, so far as it could be determined, the total amount of reduction, decrease from the axial regions basipetally. This holds not only for the single hydranths, hydranth buds, growing tips, medusa buds and stolons, but for the colony as a whole. The differences at different levels are least in the stems. The perisarc is of no very great significance as an obstacle to the passage of permanganate to the tissues within it. In the more apical regions parts inclosed in perisarc begin to stain almost or quite as quickly as naked parts, and even the thicker perisarc of the more basal regions of the stem retards the staining only slightly. The thickness of the perisarc, therefore, is certainly not responsible for the differences in rate of staining of different levels. The deposition of the oxide begins on the external surface of the protoplasm and a distinct regional gradient in staining is in many cases visible on the surface before any trace of reduction appears below the surface.

The Hydranth Gradients.—Reduction and coloration occur first at the tips of the tentacles and within a few moments each tentacle shows a distinct color gradient ranging from brown at the tip to light yellow in the basal region. Color begins to appear at the tip of the manubrium shortly after it appears on the

tentacles, never, so far as my observations go, at the same time, and progresses basipetally on the manubrium, the basal portions of the hydranth body being the last to show color. The color appears first on the external surface and it is not infrequently possible to see the formation of precipitate on the external surface of the cells. Such precipitate must result from reduction of permanganate on the external surface of the protoplasm and the reduction and coloration progress from the surface inward. The permanganate does not penetrate the living cell and kill after it attains a certain concentration in the interior but it reacts with the protoplasm and undoubtedly kills it as it comes into contact with it.

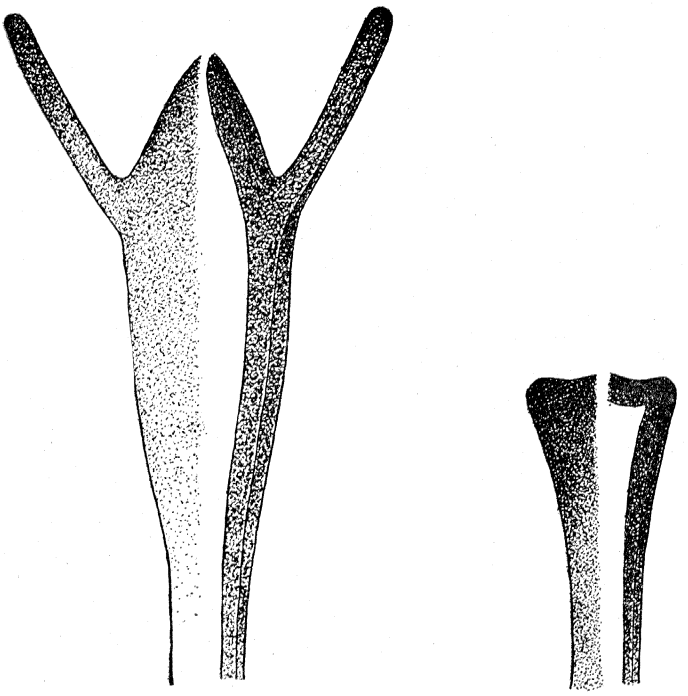
The color deepens rapidly and within fifteen minutes to an hour in concentrations ranging from $m/2000$ to $m/7500$ the regions where coloration first appeared are deep brown or opaque black and from these a color gradation to yellow in the basal regions appears.

After two to three hours the whole hydranth is usually opaque black in water. Teasing shows that at this time reduction has occurred throughout the ectoderm and that the entoderm is more or less deeply colored, and sometimes gradual differences in rate of entodermal staining can be seen, but the existence of entodermal gradients can be determined with certainty only in dehydrated and cleared material. After reduction is completed all hydranths are opaque black in transmitted light, and even after dehydration and clearing the bodies of the larger hydranths are opaque throughout, but the gradient is distinct in all tentacles and in at least the smaller hydranths.

The diagrammatic Fig. 1 indicates the color gradients in the tubularian *Bougainvillea*. The lighter side of the figure shows the gradient in rate of staining in single tentacle and the hydranth body, as seen from the surface after fifteen minutes to one hour in $KMnO_4$ according to concentration. The right side of the figure represents an optical section of tentacle and body wall after complete reduction, dehydration and clearing. The shading is intended to give some idea of the differences in depth of staining at different levels. After total reduction and clearing all parts

are very deeply colored, in fact large hydranths are often still completely opaque. The gradients are similar in the campanularian hydranth.

The Gradient in Hydranth Buds.—In *Bougainvillea* the buds show very distinct apico-basal gradients in rate of reduction from early stages on, and in material dehydrated and cleared after



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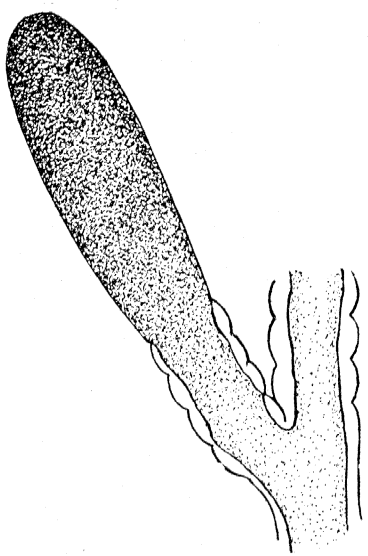
complete reduction the basipetal gradient appears in both ectoderm and entoderm. Figure 2 shows a somewhat advanced bud, the left side in surface view indicating the gradient in rate of reduction, the right side in optical section indicating the gradient in amount of reduction in cleared material.

In *Obelia*, *Gonothyræa* and other campanularians the axis of the colony is, as I have pointed out, sympodial and the bud which originated as a lateral bud below the terminal hydranth or the terminal bud undergoes considerable growth in length as a continuation of the main axis of the colony and may even give rise

to a new bud or branch before a hydranth develops at its tip (see Child, '19*b*, Fig. 10). These terminal regions of the campanularian colony and of its branches are regions of relatively rapid growth, each one representing temporarily a growing tip, and reduce $KMnO_4$ rapidly and in large amount. In spite of the fact that it is covered with thin perisarc, the coenosarc at the apical end of such a growing tip begins to stain earlier than any other part of the colony except the tentacles and in some cases the hypostome regions of hydranths. These growing tips and the hydranth buds which develop at their apical ends always show distinct basipetal gradients in rate of reduction, as indicated in Figs. 3 and 4. In preparations cleared after reduction is com-



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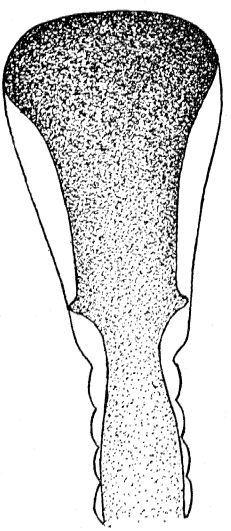

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plete these parts in vigorous, rapidly growing colonies are usually stained so deeply that they remain opaque even after clearing, but these preparations show a much greater amount of reduction in these terminal regions than in the stems.

The Gradients in Stems.—All parts of the stem are of course enclosed in perisarc which increases in thickness basipetally. Reduction of permanganate does occur to a slight extent in the

perisarc; in the thin perisarc of the more apical regions the resulting color is scarcely appreciable, but the thicker perisarc of the more basal regions, which is normally yellowish or brownish in most forms, appears more deeply colored in the permanganate, probably because of its greater thickness. In no case observed, however, is reduction in the perisarc sufficient to obscure the progress of coloration in the cœnosarc.

The permanganate penetrates the perisarc rather rapidly. The apical regions enclosed in very thin perisarc begin to stain almost as soon as the naked parts. Penetration of the thicker perisarc of the more basal regions occurs somewhat more slowly, though even in these regions the perisarc is far from being impermeable. Because of the presence of the perisarc and its different thickness at different levels, difference in rate of reduction and staining of intact stems cannot be regarded as a safe criterion of the differences in physiological condition. This difficulty, however, is easily avoided by using pieces of stems from different levels with fresh transverse cut surfaces. Pieces from different levels may be placed side by side in permanganate and the rate of coloration of the exposed distal or proximal ends directly compared. In this way it is readily determined that a gradient in rate of reaction is present in the stems. This gradient is much less steep than that of hydranths and growing tips, for reduction occurs relatively slowly in all stem regions, but with differences in level of 10–15 mm. or more it is appreciable, and between stem levels near the apical and those near the basal end of colonies several centimeters in length the difference is marked. In such cases the more apical level usually becomes distinctly yellow or even brownish before the more basal level is appreciably colored.

In preparations of whole colonies or of the longer branches, cleared after completion of reduction, the stems may remain opaque throughout if the colony is well fed and the cœnosarc thick, but in most cases a distinct basipetal decrease in opacity appears. In order to make certain that such differences are not merely apparent and due to differences in transverse diameter of the perisarc, it is desirable to compare regions from the

different levels in which the diameter of the cœnosarc is approximately the same. Repeated observations of this sort leave no doubt that real axial differences in the total amount, as well as in the rate of reduction exist in stems and branches.

The Stolon Gradient.—Many hydroid species give rise under slightly depressing conditions to stolon outgrowths, not merely from basal or even from apical cut ends, but by transformation into stolons of hydranth buds, in campanularians, of growing tips or even apical ends or stems after degeneration of hydranths. Experimental data concerning such transformations will be presented in another paper. The stolon is readily distinguishable from the stem by indefinite direction of growth, by attachment to the substratum or other solid objects and in the campanularians by absence of the annulations characteristic of stems. The stolon, like other parts of the colony, shows a gradient both in rate and amount of reduction, decreasing from the tip. This gradient is less steep than that of the hydranth or the growing tip, but steeper than that of stems. Reduction of permanganate in the growing stolon is more rapid than in the stem, but less rapid than in hydranth buds and growing tips.

The Gradient of the Colony as a Whole.—The reduction gradient appears, not merely in the individual zooids and parts of the hydroid colony but there is a general gradient characteristic of the whole colony. The gradient of stem and branches already described above is of course a part of this general gradient, but this gradient also appears, like the susceptibility gradient (Child, '19*b*) in the differences between the hydranths and growing tips of different regions.

The *Bougainvillea* colony is monopodial like *Pennaria* (Child, '19*b*) and the primary hydranth of the colony or branch remains permanently the apical hydranth. Budding occurs subapically at a certain distance below the apical hydranth, and the new axes developing from the buds become lateral branches of the colony or branch each bearing its primary apical hydranth. In any complex colonial axis, the lateral branches grow less rapidly than the main axis and the physiological relations between the different axes are apparently similar to those existing in

plants of similar growth-form, *i.e.*, the chief apical region inhibits in some way and to some extent the lateral branches. It has been shown (Child, '19*b*) that these complex axes possess a general susceptibility gradient, the apical hydranth of the main axis being in general more susceptible than the apical hydranths of lateral branches and the apical hydranths of the more apical lateral branches, more susceptible than those of more basal branches. In *Bougainvillea* this general gradient is less distinct than in some of the campanularians with more definite growth-form.

In *Obelia* and *Gonothyræa* a similar general gradient appears in the whole colony and in the complex axial systems represented by its branches. In these forms, however, the growth form is sympodial (Child, '19*b*) and instead of a permanent apical hydranth each growing tip and the hydranth bud developing from it is temporarily apical, but is displaced by the next bud. The general colony gradient appears in the rate of reduction of the different growing tips and hydranth buds of an axial complex, the most apical showing in general the most rapid reduction.

In vigorous, rapidly growing colonies of *Obelia borealis* 5 cm. or more in length, taken from piles and floats, the general gradient is usually clearly visible to the naked eye by reflected light after reduction is completed or has progressed so far that the tissues are opaque in water. On removal to water and washing the depth of brown color of the tissues of such a colony, as seen by reflected light, decreases in general from apex to base in the colony as a whole and in each axial complex. The most apical growing tips and buds are very deep brown, almost black, the growing tips and buds of more basal regions distinctly lighter in color and even in the developed hydranths similar differences appear to some extent. The color gradient in the stem also appears in spite of the fact that the thicker perisarc of the more basal regions is more deeply stained by permanganate than that of more apical regions.

It is perhaps necessary to emphasize the point that these general gradients are most distinct in vigorous, growing colonies. The observations recorded above were made on colonies which

were known to have developed within three to four weeks preceding the observations and in which medusa buds or gonozooids had begun to appear only in the basal regions. I have pointed out elsewhere (Child, '19*b*) that medusa-bud development normally begins in colonies which are relatively old physiologically and in the most basal regions of the colony, *i.e.*, in the region of least susceptibility, and as the data of the present paper show, of lowest rate of reduction of permanganate. Later, as the colony grows progressively older, medusa-buds or gonozooids appear farther apically, until finally they may develop even from the apical regions.

The old colonies with medusa-buds or gonozooids in or near the apical regions may show little or no indication of a general colony gradient. Often in such cases the original hydranths of the most basal levels have disappeared, and in some cases new young hydranths may be developing. Colonies in this stage often show a higher susceptibility and higher rate of reduction in the newly developed, physiologically younger hydranths or growing tips of the more basal regions than in those of the more apical regions, which represent the original generation. Later, when these also have been replaced by a new generation the general colony gradient may once more become like that of the young growing colony.

In short, the gradient is not a fixed unchangeable condition which can be demonstrated in any hydroid colony. It is a feature of the normal developmental and functional relations of parts, but may be altered, obliterated or even reversed by environmental or physiological conditions. The gradients in the single hydranths and growing tips are more persistent. So far as my observations go, their disappearance is followed by the disappearance through death in the case of more highly specialized, or resorption in the case of less high specialized parts. Similarly, in the colony as a whole the disappearance of the general gradient is associated with the disappearance, at least temporarily, of the original order and relation characteristic of the colony. Old hydranths or axial complexes may die or be resorbed and new ones may develop, but my observations indicate

that wherever new development of a hydranth or an orderly axial complex occurs the characteristic gradients are found to be present and after a period of resorption, redifferentiation and rejuvenescence following the stage of medusa-bud formation the general colony gradient may reappear simply as a physiological consequence of the order in which development, senescence, death or resorption, and new development occur in different parts of the colony. In other words, the new colony gradient in such cases is a physiological consequence of the existence of the original gradient. Of course, in some cases, environmental factors may play a part in altering the original relations and so in modifying the form and order of the colony.

It is of interest to note that Gast and Godlewski ('03) studying the form regulation of *Pennaria cavolinii* found a general colonial gradient in rate of regeneration of hydranths. After removal of the hydranths from a colony or axial complex, development of new hydranths decreased in rate basipetally in the colony as a whole and in each axial complex. Later degeneration of the regenerated hydranths began in the most basal regions and proceeded a greater or less distance apically, but the most apical hydranths persisted, grew and budded at the expense of these other more basal parts. Both of these gradients, the regeneration gradient and the degeneration gradient, indicate that the fundamental physiological activity of the protoplasm is greater apically and decreases basipetally in the colony as a whole and in each axial complex. In short, these regulatory phenomena constitute still another line of evidence for the existence of the physiological gradients.

And finally, I am permitted to state from unpublished work that Dr. A. W. Bellamy and Dr. L. H. Hyman, working independently, have found gradients in electric potential in hydroid colonies of various species. These electrical gradients correspond to the gradients in susceptibility, reduction of permanganate and rate of regulation. In vigorous, growing colonies the apical region of the colony is galvanometrically negative to all other levels, the apical region of each axial complex is negative to all other parts of that complex, and the apical regions of the

more apical branches are negative to the apical regions of the more basal branches.

The Gradient of the Medusa Bud.—In the naked medusa buds of *Bougainvillea* and other tubularians the gradients in rate and amount of reduction of $KMnO_4$ appear very distinctly. The free apical end of the bud always shows the highest rate and greatest amount of reduction, as indicated in Fig. 5, a young bud of *Bougainvillea*. The medusa buds of the campanularians constitute less favorable material because the crowding along the blastostyle and the presence of the gonotheca render it impossible to insure uniform concentration of permanganate over all parts of the surface. In general, however, it is perfectly clear that the apical regions of these buds reduce permanganate more rapidly and in greater amount than other levels.

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In *Gonothyræa* the rudimentary medusæ or gonophores in the later stages of development come to lie outside the gonotheca, though still attached to the blastostyle, and so are directly exposed to permanganate solutions. The rate of reduction is highest in the small tentacles of these gonophores and decreases basipetally over the outer surface of the body. The subumbrellar cavity of the gonophore is at first closed and never widely open to the exterior, so that it is not possible to determine with certainty the relative rate of reduction in this region, though it is probably much the same as in other medusæ. In the old gonophores after extrusion of the planulæ the reduction gradient may be almost or entirely absent.

Reduction Gradients in Free-living Hydromedusæ.

The simple experiment of placing medusæ in a permanganate solution and agitating sufficiently to provide for uniform distribution of the permanganate was performed repeatedly during three summers and always with essentially the same result. In all forms examined reduction occurs most rapidly in the marginal tentacles and the oral lobes or tentacles, when such are present. Each tentacle or lobe shows a gradient in rate of reduction,

decreasing basipetally. Elongated manubria show similar gradients in rate of reduction. Manubrium and margin are regions of more rapid reduction than other subumbrellar regions, but the subumbrellar ectoderm in general reduces more rapidly than the exumbrellar ectoderm. Dehydration and clearing is not necessary in the medusa species used because the cellular tissues are mostly in such thin layers that they do not become opaque black. Nevertheless, the final difference in depth of color between exumbrella and subumbrella cannot be regarded as a fair criterion of differences in total amount of reduction because the thickness of cellular tissue is much greater in the subumbrella than in the exumbrella. On this account I am inclined to regard the differences in rate of reduction as more significant than the final differences in depth of color. The jelly reduces little permanganate and reduction is very slow.

Data on susceptibility of medusæ have not previously been recorded, but numerous experiments have been performed, chiefly on *Æquorea*, but in part on other species, with various concentrations of several agents, viz., KNC, *m*/100–*m*/500, HCl, *m*/200–*m*/800 and KOH, *m*/200–*m*/600. The susceptibility gradients in all these agents are essentially the same as the reduction gradients. A few experiments with neutral red and methylene blue and various other agents on the smaller forms gave similar results. It may be said then that here, as in the hydroids, the susceptibility gradients and the reduction gradients correspond.

It is of interest to note that the higher susceptibility and more rapid reduction of the subumbrellar as compared with the exumbrellar regions is in accord with McClendon's data on oxygen consumption in *Cassiopea*. He found that the subumbrella consumed much more oxygen per unit of weight than the exumbrella plus the greater part of the mesoglœa (McClendon, '17). Dr. Bellamy and Dr. Hyman have found differences in electric potential corresponding to the differences in susceptibility and rate of reduction, not only as regards subumbrella and exumbrella, but also as regards marginal and oral, as compared with other subumbrellar regions. The regions of highest galvano-

metric negativity correspond in all cases to the regions of highest susceptibility and rate of reduction.

Reduction after Killing.

The gradients in rate and amount of reduction of permanganate appear when the living animals are brought into permanganate, but when they are first killed by some other means and then brought into permanganate the gradients are either entirely absent, or only the merest traces of them remain. Various means of killing have been used in these experiments, among them $HgCl_2$, $HgCl_2$ with 5 per cent. acetic acid, HNO_3, HCl, strong alcohol, formalin, hot sea water. If the animals are brought into permanganate within a few moments after the killing agent is applied, with only a brief washing in sea water, if necessary to remove excess of the killing agent, slight gradients may still appear. So far as my observations go, however, this is not the case if the animals are subjected after killing to the usual treatment of histological material. If, for example, they are passed through the alcohols up to 70–80 per cent. and kept in this percentage for a day or two, or if they are killed and kept in formalin and then washed in water before being placed in permanganate, the rate of reduction in different parts is uniform and the only differences in depth of color, so far as could be determined, are the apparent differences resulting from differences in thickness of the layers through which the light passes. Moreover, in these killed animals the total amount of reduction is much less than when the living animals are brought into permanganate. Potassium permanganate, even in high dilutions, is highly toxic and when the living animals are placed in it they are of course killed by it, but it is evident from the facts cited that the differences in physiological condition in different regions play a part in determining the rate and amount of reduction. In the dead animals these differences are no longer present, although some factor or factors concerned in them may persist for a time after the killing agent is applied. Whatever the nature of the differences in reaction to permanganate between living and dead protoplasm, it is evident that the gradients in

rate and amount of reduction are associated with and dependent upon the living condition and disappear, either with, or soon after, death.

Conclusion.

Since $KMnO_4$ is a powerful oxidizing agent, the inference is justified, as I have pointed out elsewhere (Child, '19*a*), that the rate and amount of reduction of permanganate by living protoplasm is in some way and to some degree related to oxidative activity in the protoplasm. This inference is apparently supported by the marked decrease in reducing capacity of the protoplasm with death and the disappearance with, or soon after death, of the regional differences or gradients in rate and amount of reduction. Moreover, the reduction gradients are essentially the same as the susceptibility gradients and many different lines of evidence indicate that the latter are, at least to some extent, associated with and dependent upon differences in rate of oxidation (Child, '20). The results obtained with permanganate are then in complete agreement, as regards the existence of physiological gradients, with those obtained by other methods and constitute another line of evidence in support of the general conception.

The question of the rôle of permeability in relation to the reduction gradients must be raised. It has been repeatedly pointed out (Child and Hyman, '19, Child, '20) that gradients in permeability are in many, if not in all cases, characteristic features of the physiological gradients, but many facts show clearly enough that the fundamental metabolic activity of the protoplasm is also concerned in these gradients. We are forced to conclude, either that the gradients are not simply permeability gradients, or else that permeability and the fundamental metabolism of the protoplasm are very intimately associated and more or less interdependent. As regards the reduction gradients, it may seem at first glance that the differences in rate of reduction are merely the result of differences in permeability to permanganate. This is perhaps to some extent the case, but observation indicates that these differences appear first upon the external surface of the protoplasm. It is not necessary that the per-

manganate shall penetrate to the interior of the cell before the reaction begins. Moreover, the protoplasm in which the reaction occurs is undoubtedly killed very rapidly, as is indicated by the almost instantaneous cessation of ciliary and flagellar activity, even in very low concentrations of permanganate. It is certain that the permanganate does not penetrate the plasma membrane or any other limiting surface to any appreciable extent while that surface is living, but rather kills or begins killing as it comes into contact with the surfaces. My observations indicate, however, that the differences in rate of reduction disappear after death. If this is true, permeability in any physiological sense, *i.e.*, permeability of living protoplasmic limiting surfaces, cannot be the chief factor in determining the gradients in rate of reduction, for any differences in such permeability must disappear very soon on contact with the solution. If the particular limiting surfaces persist as limiting surfaces after the action of permanganate, and it is by no means certain to what extent they do persist, their permeability is no longer the physiological permeability of the living surface, but simply that of a dead surface. In short, after a given limiting surface, whether external or internal, has been killed by permanganate, the further passage of that surface by permanganate is not determined by its physiological permeability. If this argument is correct, the differences in rate of reduction of permanganate determined by differences in physiological permeability should be slight, since permanganate is highly toxic, but, as a matter of fact, the differences are very great when living animals are brought into the solutions and absent, or almost absent when dead animals are used.

Moreover, the deposition of MnO_2 or other oxides in the protoplasm is the result of a chemical reaction between the permanganate and protoplasmic constituents and therefore depends on other factors than the mere entrance of the agent. There is every reason to believe that the chemical and particularly the oxidative activity of the protoplasm is a factor in determining the velocity of the reaction, even though death occurs rapidly. Exactly what changes constitute death in permanganate, or for that matter in any other agent, it is impossible to say, but it is

evident that the beginning of death does not stop the reduction of permanganate, and even after death is complete the reaction occurs to some extent. The slight regional differences in rate of reduction which sometimes persist for a time after death is apparently complete may perhaps mean that certain death changes, *e.g.*, inactivation of oxidizing enzymes, stabilization of oxidizable substance, are not yet complete, but at most these differences are mere vestiges of the original differences.

And finally, the regional differences in the total amount of reduction cannot be due to differences in the permeability of limiting surfaces. In the light of all the facts concerning the physiological gradients, the only conclusion at present justifiable seems to be that they are associated with and dependent upon differences in the chemical, and particularly the oxidative activity of the protoplasm concerned, though it is, of course, granted that various factors are concerned in these chemical differences. The reduction gradients appear only when living protoplasm reacts with permanganate, or as mere vestiges in protoplasm very recently killed. The regions of greatest amount of reduction are the regions of greatest physiological activity, as indicated by growth, development, susceptibility, electro-negativity, and in those forms for which it has been determined, of respiratory activity. I believe we are justified in concluding that, when used with proper precaution and in connection with other methods, the rate and amount of reduction of potassium permanganate by organisms which are alive at the beginning of the reaction serves as an indicator of the fundamental physiological condition of the protoplasm. This conclusion holds, not only for hydroids, but for all other forms examined (Child, '19*a*).

Summary.

Hydroids and hydromedusæ placed in dilute solutions of potassium permanganate show characteristic axial gradients in rate and total amount of reduction of permanganate, as indicated by the coloration of the protoplasm by MnO_2 or other products of the reaction. These gradients correspond with the physiological gradients indicated by other methods and by the development, growth-form and functional activities of the organisms.

The gradients are present, not only in each zooid of the hydroid colony and in each tentacle of the hydranth, but in each axial complex, *e.g.*, a compound branch, and in the colony as a whole, provided it is vigorous and growing. In general the rate and amount of reduction decrease basipetally. In the medusæ reduction is most rapid and greatest in amount in the marginal and oral regions, less in other subumbrellar regions and least in the exumbrellar region. Animals killed by other means before reaction with permanganate show no gradient, or in some cases vestiges, if newly killed. The conclusion is drawn that differences in rate and amount of reduction of permanganate, as indicated by the coloration, may, with proper precautions, be used as an indicator of differences in fundamental physiological condition in different regions of an organism.

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